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IV. SYNTHESIS OF AN AFFINITY GEL FOR PURIFICATION OF BETA-GLUCOSIDASES

Karel Grohmann, Ken K. Oh and Michael E. Himmel

INTRODUCTION

As observed in Section II, the purification of beta-glucosidases from spent fungal or bacterial growth media can be a multi-step operation requiring months of effort. Also, the limiting quantity of beta-glucosidase compared to other proteins present in growth supernatants and cell lysates makes classical purification (i.e., repetitive gel filtration and ion-exchange chromatography) difficult and ineffective. Late in FY 1987 we examined the applicability of an immobilized substrate-analog affinity approach to purification of these enzymes.

EXPERIMENTAL, RESULTS AND DISCUSSION

Para-aminophenyl-1-thio-beta-D-glucopyranoside (compound I) was prepared following the methods of (Iino et al, 1976 and Chipowsky et al, 1973). Here 2,3,4,6-tetra-o-acetyl-alpha-D-glucopyranosyl bromide was condensed with p-aminobenzenethiol hydroxide (See reaction scheme in Figure 1). The reaction was completed in four hours at room temperature. The acetone was evaporated and the precipitated intermediate product (p-aminophenyl 2,3,4,6-tetra-o-acetyl-1-thio-beta-D-glucopyranoside) was washed with dilute (1%) aqueous sodium carbonate solution to remove unreacted p-aminobenzenethiol. The intermediate compound was deacetylated by sodium methoxide in methanol. Excess base was removed by addition of Dowex 50-X8 ion-exchange resin. The dark brown solution was partially decolorized with activated charcoal and methanol was evaporated. The p-aminophenyl-1-thio-beta-D-glucopyranoside was then dissolved in water and coupled to Affigel 10 activated resin (BioRad Labs) per manufacturer's recommendations. To test the activated resin, a sample of NOVO SP188 beta-glucosidase containing 6 OD (280 nm) was dialyzed against 20 mM acetate buffer pH 5 and loaded onto a 50 mL column of resin.

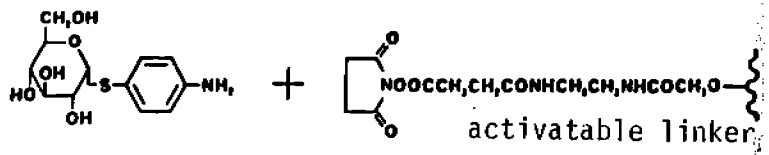
Figure 1 shows the results of the affinity chromatography using the activated resin. The first peak shown corresponded to 8% of the activity loaded on the column. Following the elution of this breakthrough peak, the column was washed extensively (approx. 20 column volumes) with loading buffer. The second peak shown in the figure eluted during a 0 - 0.5 M NaCl gradient. This peak proved upon assay to contain all of the remaining beta-glucosidase activity originally loaded. The activity which eluted from the column immediately after loading may have resulted from over-saturation of the binding sites on the column. Regardless, the affinity method achieved a 10 fold purification (e.g., 0.6 OD beta-G from 6 OD starting material) in one step. The level of purity estimated from HPSEC and SDS-PAGE was about 95%.

REFERENCES

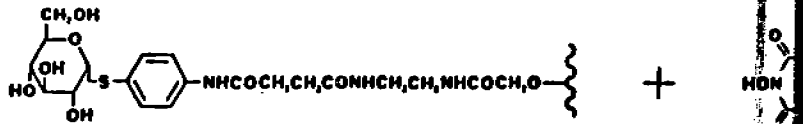
Chipowsky, S., and Lee, Y.-C. 1973, Carb. Res. 31, 339-346.

Iino, N., and Yoshida, K. 1976, Carb. Res. 51, 223-228.

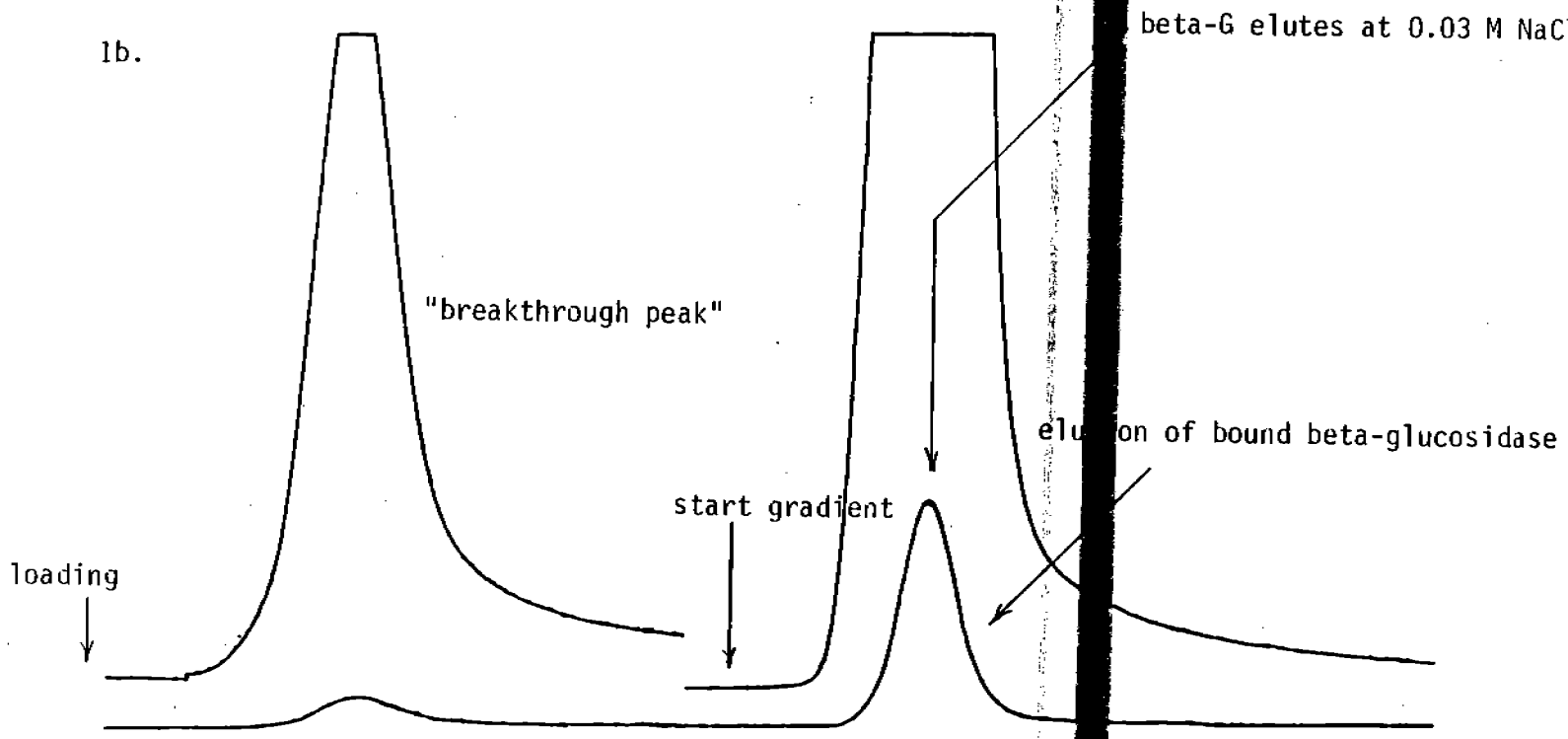
1a.


activatable linker

(I)


coupled resin

1b.


beta-G elutes at 0.03 M NaCl
"breakthrough peak"
elution of bound beta-glucosidase
start gradient
loading